Beamline 5.0.2

Multiple-Wavelength Anomalous Diffraction (MAD) and Monochromatic Protein Crystallography

Cocrystal structure determination of a pseudouridine synthase, a nucleotide-flipping RNA-modifying enzyme, at ALS Beamline 5.0.2
Hoang, C., A.R. Ferré-D'Amaré

Crystal structure of nitric oxide synthase bound to nitroindazole reveals a novel inactivation mechanism
Raman, C.S., H. Li, P. Martásek, G. Southan, B.S.S. Masters, T.L. Poulos

Structural studies of chromosomal building blocks
Chakravarthy, S., R. Edayathumangalam, U.M. Muthurajan, R.K. Suto, C.L. White, K. Luger

The high-resolution structure of the RNase P protein from *Thermotoga maritima* reveals a remarkable similarity among bacterial RNase P proteins
Kazantsev, A.V., A.A. Krivenko, D.J. Harrington, R.J. Carter, S.R. Holbrook, P.D. Adams, N.R. Pace

COCRYSTAL STRUCTURE DETERMINATION OF A PSEUDOURIDINE SYNTHASE, A NUCLEOTIDE-FLIPPING RNA-MODIFYING ENZYME, AT ALS BEAMLINE 5.0.2

C. Hoang and A.R. Ferré-D'Amaré
Division of Basic Sciences, Fred Hutchinson Cancer Research Center,
1100 Fairview Avenue North, Seattle, WA 98109-1024, USA

SUMMARY

Pseudouridine (Ψ) synthases catalyze the isomerization of specific uridines in cellular RNAs to pseudouridines and may function as RNA chaperones. TruB is responsible for the Ψ residue present in the T loops of virtually all tRNAs in the cell. The close eukaryotic homolog Cbf5/dyskerin is the catalytic subunit of box H/ACA snoRNPs that catalyze modification of ribosomal RNA. The 1.85 Å resolution structure of TruB bound to an inhibitor RNA [1] shows that this enzyme recognizes the preformed three-dimensional structure of the T loop. It gains access to its substrate uridyl residue by flipping out the nucleotide, and thus disrupts tertiary, or long-range, interactions between the T and D loops of tRNA. This is the first structure determination of an RNA base-modifying enzyme bound to a substrate.

BACKGROUND

Pseudouridine (Ψ) is the most abundant modified nucleotide in RNA. The isomerization of U into Ψ involves breakage of the glycosidic bond connecting the ribose to the pyrimidine base, rotation of the detached base, and reconnection through C5 (Fig.1). These enzymes require no cofactors or external sources of energy.

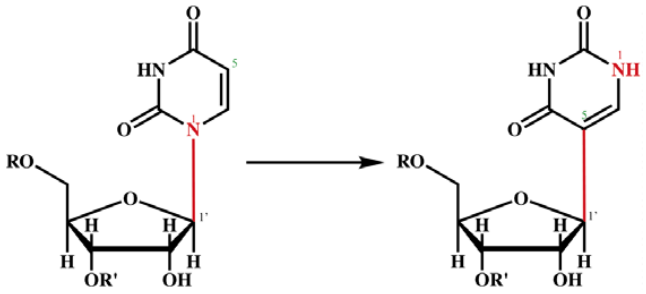

Figure 1. Isomerization of uridine (left) into Ψ catalyzed by Ψ synthases. Note how the glycosidic bond (in red) in uridine is replaced by a carbon/carbon bond in Ψ. R and R' denote the RNA chain to either side of the site of modification.

EXPERIMENTAL DESIGN

In order to understand how a Ψ synthase recognizes its target RNA, and how the post-transcriptional catalytic transformation is achieved, we determined the structure of *Escherichia coli* TruB in complex with an RNA-based inhibitor (Fig. 2A). RNAs with a 5-fluorouracil (5FU) residue at the site of modification are tight mechanism-based inhibitors of tRNA Ψ synthases [2]. Since the regioselectivity and kinetics of pseudouridylation are the same on full-length tRNAs and RNAs comprised of the isolated T stem and loop (TSL RNAs), all determinants of specific Ψ55 synthase-tRNA recognition must lie within this segment of tRNAs [3]. A 22-nucleotide (nt) TSL with 5FU at the site of modification produced well-ordered cocrystals. The TruB cocrystal structure reveals that this Ψ synthase gains access to its substrate by flipping out nucleotide 55 of tRNA. In the TruB-TSL complex, the bases of nucleotides 55, 56, and 57 are everted from the position they would assume within the helical stack of isolated, folded tRNA (Fig. 2B). In folded tRNAs, these three nucleotides in the T loop contact the D loop to stabilize the tertiary structure of the RNA.

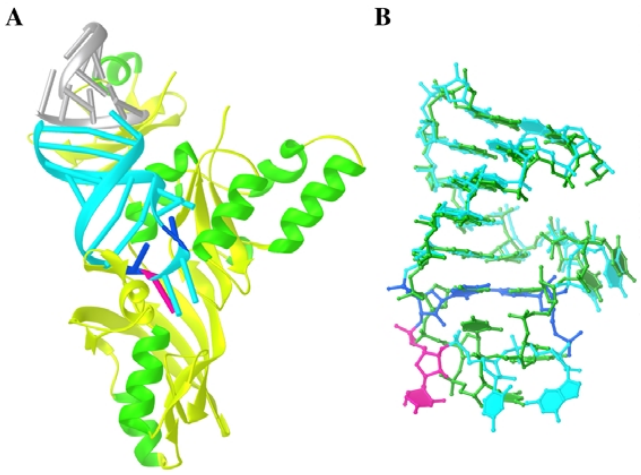

Figure 2. Three-dimensional structure of the Ψ synthase TruB complexed to a T stem-loop RNA. **A**.Ribbon representation of the complex. Protein helices are dark green, strands and loops are light green. The TSL RNA is light blue, except for nucleotides U54 and A58 that make a conserved reverse Hoogsteen pair colored dark blue, and U55 (the site of pseudouridylation) in purple. A segment of a symmetry-related RNA that extends the A-form helix is shown in light gray. **B.** Superposition of the TSL bound to TruB (colored as in Figure 1) with the corresponding residues from the structure of intact, folded $tRNA^{Phe}$ (in green). Three nucleotides at the apex of the T loop are flipped out of the helical stack by binding to TruB.

STUDIES CONDUCTED AT THE ADVANCED LIGHT SOURCE

Our TruB cocrystals have the symmetry of space group *C*2, and unit cell dimensions $a = 145.05$ Å, $b = 40.36$ Å, $c = 77.99$ Å and $\beta = 110.60°$. Experimental phases were calculated at 2.0 Å resolution using MAD data measured at beamline 5.0.2 using a cocrystal containing a selenomethionine version of TruB. The MAD diffraction data were collected at three wavelengths corresponding to the peak, inflection point, and high energy remote for selenium as determined from a flurorescence energy scan of the actual crystal. Given the low symmetry of the crystal, in order to obtain complete anomalous data sets, 360° of data were collected at the both the peak and remote wavelengths. A 180° of data were collected at the inflection point. The three X-ray energies remained very stable over the eleven hours of data collection. 'Solvent flattening' and phase extension to 1.85 Å resolution produced an experimental electron density

map into which most of the protein and RNA residues could be built unambiguously (Fig. 3). The structure has been refined to an *R* factor of 18.4% and a free *R* factor of 21.2%.

The cocrystal structure of TruB reveals for the first time how a Ψ synthase recognizes its substrate and suggests how it may function in promoting RNA folding [1]. This high resolution structure is the starting point for unravelling the mechanism of action of these phylogenetically conserved enzymes, and also for exploring their possible roles in maturation and assembly of RNAs.

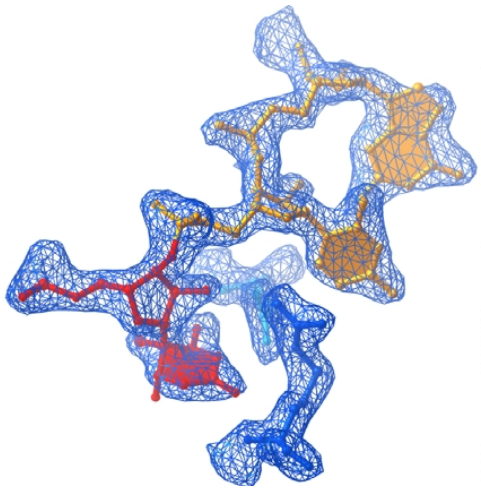

Figure 3. Portion of the 1.85 Å resolution 'solvent flattened' MAD experimental electron density map corresponding to part of the active site of TruB, contoured at 1.5 standard deviations above mean peak height.

ACKNOWLEDGMENTS

We thank T. Earnest and K. Henderson for help at Advanced Light Source (ALS) beamline 5.0.2; J. Bolduc, P. Heath and B. Shen for crystallographic and computational support; and S. Biggins, D. Gottschling, H. Houbaivy, M. Roth, P. Rupert, S. Sigurdsson, J. Simon, B. Stoddard, G. Varani and D. Wilson for discussions.

REFERENCES

1. C. Hoang and A.R. Ferré-D'Amaré, "Cocrystal structure of a tRNA Ψ55 pseudouridine synthase: nucleotide flipping by an RNA-modifying enzyme," Cell **107,** 929 (2001).
2. T. Samuelsson, "Interactions of transfer RNA pseudouridine synthases with RNAs substituted with fluorouracil," Nucleic Acids Res. **19**, 6139 (1991).
3. X. Gu, M. Yu, K. M. Ivanetich and D.V. Santi, "Molecular Recognition of tRNA by tRNA pseudouridine 55 synthase," Biochemisty **37,** 339 (1998).

This work was supported by grants from the National Institutes of Health and the Rita Allen Foundation to A.R.F., and from the American Cancer Society to the Fred Hutchinson Cancer Research Center (FHCRC). Access to ALS beamline 5.0.2 as part of the principal research consortium was made possible by general support from the FHCRC. A.R.F. is a Rita Allen Foundation scholar.

Principal Investigator: Adrian R. Ferré-D'Amaré, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, WA 98109-1024. Email: aferre@fhcrc.org. Telephone: 206-667-3622.

Crystal structure of nitric oxide synthase bound to nitroindazole reveals a novel inactivation mechanism

C. S. Raman,[*] Huiying Li,[*] Pavel Martásek,[†] Garry Southan,[¶]
Bettie Sue S. Masters,[†] and Thomas L. Poulos[*]

[*]Departments of Molecular Biology & Biochemistry and Physiology & Biophysics and the Program in Macromolecular Structure, University of California, Irvine, California 92697

[†]Department of Biochemistry, University of Texas Health Science Center, San Antonio, Texas 78229

[¶]Inotek Corp., Beverly, MA 01915.

Nitric oxide is generated under normal and pathophysiological conditions by three distinct isoforms of nitric oxide synthase (NOS). A small-molecule inhibitor of NOS (3-Br-7-nitroindazole, 7-NIBr) is profoundly neuroprotective in mouse models of stroke and Parkinson's disease. We report the crystal structure of the catalytic heme domain of endothelial NOS complexed with 7-NIBr at 1.65 Å resolution. We also present two crystal structures of eNOS complexed with either 7-nitroindazole-2-carboxamidiine or N-(4-chlorophenyl)-N'-hydroxyguanidine that reveal how alterations at the substrate site facilitate 7-NIBr and structurally dissimilar ligands to occupy the cofactor site. The x-ray diffraction data reported here were in part collected at Beamline 5.0.2 at ALS. Critical to the binding of 7-NIBr at the substrate site is the adoption by eNOS of an alternate conformation, in which a key substrate binding residue, Glu-363, swings out toward one of the heme propionate groups. Perturbation of the heme propionate ensues and eliminates the cofactor tetrahydrobiopterin-heme interaction. In fact, 7-NIBr selects for the catalytically incompetent Glu-363 rotamer and by binding to the substrate site locks this conformation in place. By competing simultaneously for both the substrate and cofactor binding sites, 7-NIBr is able to occupy one site and subsequently alter the specificity of a second site. Structural analyses of the 7-NIBr-bound eNOS structure teaches us that designing an inhibitor, which avoids H-bonded contact with one of the heme propionates and concurrently selects for the alternate Glu-363 rotamer, can serve as a potential template for designing drugs with isoform specificity. This is because such compounds dramatically weaken the affinity of the cofactor site for H_4B and subsequently make it promiscuous. Therefore, one can take advantage of the small but significant differences at the substrate and cofactor binding sites toward designing bifunctional drugs with isoform selectivity.

This work was supported by NIH grants GM 57353 (TLP), and GM52419 (BSSM) and Robert A. Welch Grant No. AQ-1192 (BSSM).

Contact person: Dr. Huiying Li, Dept. MB&B UCI Tel: (949)-824-4322 Email: hli@bragg.bio.uci.edu

Structural studies of chromosomal building blocks

Srinivas Chakravarthy, Rajeswari Edayathumangalam, Uma M. Muthurajan, Robert K. Suto, Cindy L. White, Karolin Luger
Colorado State University (CSU), Department of Molecular Biology, Fort Collins, CO-80523-1870

INTRODUCTION

The fate of a multi-cellular organism depends on the timed and coordinated readout of its genes. At the molecular level, this requires the accurate transcription of a subset of genes from the total complement that is present in each cell. Consequently, certain diseases and developmental disorders are associated with and even caused by aberrant gene expression. The DNA of a single mammalian cell is over two meters in length, but compacts in the cell nucleus to nearly one millionth of this dimension by a hierarchical scheme of folding and compaction into a highly dynamic protein-DNA assembly termed chromatin. Activation of a gene requires its identification within compacted chromatin. Local unpacking and remodeling of chromatin allows access of regulatory proteins and the transcription machinery, resulting in gene activation. Thus, the organization of DNA in chromatin has profound implications for the regulation of gene expression.

High-resolution crystal structures of nucleosome core particles (NCP) from *Xenopus laevis* reveal an octameric histone core around which 147 base pairs of DNA are wrapped in 1.65 superhelical turns [1] (Fig. 1). The histone octamer itself is composed of two copies each of the four histone proteins H2A, H2B, H3, and H4. Massive distortion of the DNA is brought about by the tight interaction between the rigid framework of the histone proteins with the DNA at fourteen independent DNA binding locations [1, 2]. We have previously determined the structure of a nucleosome containing an essential histone variant, from data collected at the ALS [3]. These studies are now being extended to study nucleosomes from other organisms, and are being expanded into new areas.

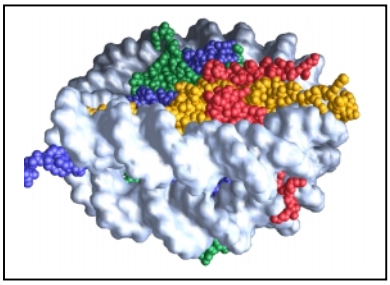

Fig. 1: Structure of the NCP. DNA is shown as a molecular surface in white, protein is shown as a space-filling model in red (H2B), yellow (H2A), blue (H3), and green (H4).

STRUCTURE OF THE YEAST NUCLEOSOME CORE PARTICLE

Fundamental differences between the yeast genome and that of higher organisms suggest that chromatin might be organized in a different manner in yeast. Yeast is a unicellular organism whose entire genome is only ~ 0.5 % the size of that of humans, and its histone proteins are the most divergent among all eukaryotes. The crystal structure of the nucleosome core particle from *Saccharomyces cerevisiae* reveals that the structure and function of this fundamental complex is conserved between single-cell organisms and metazoans [4]. Our results show that yeast nucleosomes are likely to be subtly destabilized as compared to nucleosomes from higher eukaryotes, consistent with the notion that much of the yeast genome remains constitutively open during much of its life cycle. Importantly, minor sequence variations lead to dramatic changes in the way in which nucleosomes pack against each other within the crystal lattice. This has important implications for our understanding of the formation of higher-order chromatin structure and its modulation by post-translational modifications. Finally, the yeast nucleosome core particle provides a structural context by which to interpret genetic data obtained from yeast. Coordinates have been deposited with the Protein Data Bank under accession number 1ID3.

SITE-SPECIFIC RECOGNITION OF NUCLEOSOMAL DNA

The ability of a sequence-specific DNA binding protein to recognize its cognate site in chromatin is restricted by the structure and dynamics of nucleosomal DNA, and by the translational and rotational position of the histone octamer with respect to the binding site. Here we use high-affinity, sequence-specific pyrrole-imidazole polyamides as molecular probes for DNA accessibility in nucleosomes. Sites on nucleosomal DNA facing away from the histone octamer, or even partially facing the histone octamer, are fully accessible and the nucleosomes remain fully folded upon binding. Polyamides only fail to bind where sites are completely blocked by interactions with the histone octamer.

We have determined several high-resolution crystal structures of nucleosome core particles in complex with different hairpin pyrrole-imidazole polyamides (Fig. 2). These structures represent the first nucleosome – ligand co-crystal structures, and provide the first insight into the molecular details of base-specific DNA recognition of nucleosomal DNA. We showed that the binding of ligand does not disrupt any interactions between histones and DNA [5]. All polyamides fit snugly in the minor groove of nucleosomal DNA. Extensive hydrogen bonding between ligand and bases (in addition to non-specific hydrophobic interactions) accounts for the observed high specificity of binding, according to the specificity rules stated by Dervan and colleagues [6]. Local structural changes are imparted on the nucleosomal DNA upon binding. The ligand-induced changes in DNA topology are compensated for by conformational changes in the DNA that are distant from the binding site.

The observed effects of complex formation on the structure of polyamides and nucleosomal DNA have implications for the binding of sequence-specific transcription factors to nucleosomal DNA, and demonstrate a surprising flexibility and plasticity of nucleosomal DNA. Our results demonstrate that much of the DNA in the nucleosome is freely accessible for molecular recognition.

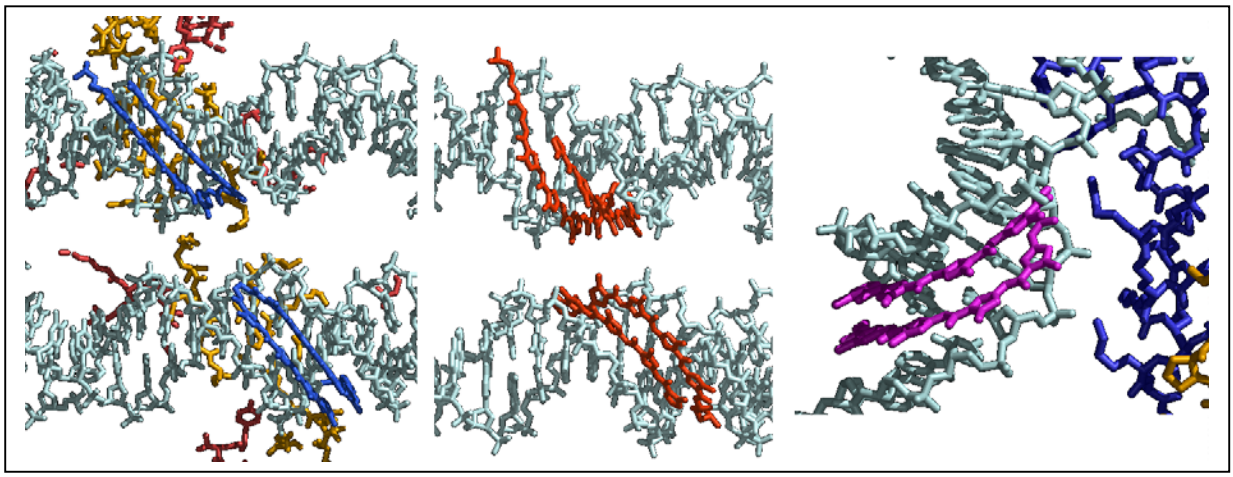

Fig. 2: Close-up view of three NCP-polyamide co-crystal structures. View is from the outside of the nucleosome. Polyamides with different sequence specificities are shown in blue, orange, and magenta, respectively.

This work was funded by grants from the NIH (GM61909), March of Dimes (99-853), Human Frontiers Programs Organization (00059), and by the Searle Scholar award to KL.

Principal Investigator: Karolin Luger, Colorado State University, 970-491-6405, kluger@lamar.colostate.edu.

REFERENCES:

1 Luger, K. *et al*. (1997) *Nature* 389, 251-259

2 Luger, K. and Richmond, T. J. (1998) *Current Opinion in Structural Biology* 8, 33-40

3 Suto, R. K., Clarkson, M. J., Tremethick, D. J. and Luger, K. (2000) *Nat Struct Biol* 7, 1121-1124

4 White, C. L., Suto, R. K. and Luger, K. (2001) *Embo J* 20, 5207-18.

5 Gottesfeld, J. M. *et al*. (2001) *J Mol Biol* 309, 625-39.

6 Dervan, P. B. (2001) *Bioorg Med Chem* 9, 2215-35.

The high-resolution structure of the RNase P protein from *Thermotoga maritima* reveals a remarkable similarity among bacterial RNase P proteins.

Alexei V. Kazantsev[*], *Angelika A. Krivenko*[*], *Daniel J. Harrington*[†], *Richard J. Carter*[‡], *Stephen R. Holbrook*[‡], *Paul D. Adams*[‡] *and Norman R. Pace*[*].

[*]*Department of MCD Biology, University of Colorado, Boulder, CO 80302*

[†]*Stanford Synchrotron Radiation Laboratory, Stanford University, Menlo Park, CA 94025*

[‡]*Physical Biosciences Division, Lawrence Berkeley National Lab, Berkeley, CA 94720*

RNase P, the ubiquitous endonuclease that catalyzes maturation of the 5'-end of tRNA, in Bacteria is a ribonucleoprotein particle composed of a large, catalytically active RNA and a small protein. Two major structural classes of RNase P RNA have been identified by phylogenetic comparative analysis, the A (ancestral) and B (Bacillus) types[1]. We have solved the X-ray crystal structure of the RNase P protein from the hyperthermophilic bacterium *T.maritima* at 1.3Å resolution[2]. This protein binds the A-type RNase P RNA.

In the crystal, the protein forms asymmetric dimers. In the light of substantial differences in the A and B-type RNAs, this protein structure bears remarkable similarity to the recently determined structures of RNase P proteins from organisms with B-type RNAs[3]. Structural conservation of potential RNA-binding elements in RNase P protein indicates that it binds to RNase P RNA primarily via direct contacts with the phylogenetically conserved core that is shared by A and B-classes of RNase P RNA.

In order to assess a potential physiological role for the protein dimerization, attempts to detect the dimerization in solution by light-scattering technique are currently in progress.

REFERENCES

1. Frank D.R. and Pace N.R, 1998. Ribonuclease P: unity and diversity in a tRNA processing ribozyme. *Annu Rev Biochem* **67**:153-180.
2. Kazantsev, A.V. et al., The high-resolution structure of the RNase P protein from *Thermotoga maritima* reveals a remarkable similarity among bacterial RNase P proteins (in preparation).
3. Stams T. et al., 1998. Ribonuclease P protein structure: evolutionary origins in the translational apparatus. *Science* **280**:752-755.

This work has been supported by NIH grant GM34527.

Principal investigator: Norman R. Pace, Department of MCD Biology, University of Colorado, Boulder, CO 80302. Email: nrpace@colorado.edu. Telephone: 303-735-1808.